

INDUCED HOST ODOR ATTRACTION IN THE PEA CRAB *PINNOTHERES MACULATUS*

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Chemoreception often plays an important part in the initiation and maintenance of marine symbiotic relationships (Davenport, 1955, 1966; Dales, 1966; Castro, 1978), including pinnotherid decapod crustaceans (*Dissodactylus mellitae*: Johnson, 1952; Gray, McCloskey, and Weihe, 1968; *Pinnixa chaetoptera*: Davenport, Camougis, and Hickok, 1960; *Pinnotheres maculatus*: Sastry and Menzel, 1962; *Opisthopus transversus*: Webster, 1968). Sastry and Menzel (1962) found that adult specimens of *P. maculatus* removed from the bay scallop *Argopecten irradians concentricus* are attracted to the odor of live specimens of both *Argopecten* and *Atrina rigida* (pen shell), but not to odor from their shells alone. This implies that the attractant is a metabolic product of the living host. Other sensory factors may be important in the associations of pinnotherids with their hosts. Phototactic responses may direct larvae of *P. maculatus* (Welsh, 1932) and other pinnotherids (Miyake, 1935) toward their hosts at the end of the planktonic stage. Rheotaxis and thigmotaxis can be used by adult specimens of *P. maculatus* in host location (Eidemiller, 1969).

Host location is important at several stages in the life cycle of *P. maculatus*. The planktonic zoeal and megalopal stages are followed by a first crab stage ("invasive stage"), at which time crabs leave the plankton and invade hosts. Following several molts within the host, the "hard stage" develops. Males and females of this stage leave their hosts, engage in an open water copulatory swarming, resettle into hosts, and pass through four posthard stages before reaching the adult instar (Pearce, 1964, 1966). Host location would therefore be necessary at the invasive and hard stages, as well as at any stage when the host might die. There are fourteen reported host species for *P. maculatus*; of these, ten are bivalve species, including the blue mussel *Mytilus edulis*, the bay scallop *Argopecten irradians concentricus*, the ocean scallop *Placopecten magellanicus*, the horse mussel *Modiolus modiolus*, and *Modiolus americanus* (Williams, 1965).

Search images are said to be involved in selective predation (Tinbergen, 1960). Search images are formed by a predator following recent encounters with a prey species, resulting in an improved ability of the predator to detect that prey species (Krebs, 1973). Search images may involve selective attention for certain stimulus objects (Curio, 1976). Although work has concentrated on visual predators, there is evidence that chemical search images may be developed by predators that use their chemical senses to a greater degree (Atema, 1977, 1980). Search images may also be used in symbiotic associations by a symbiont that must actively search for its host.

The present investigation examines chemically mediated host location in *Pinnotheres maculatus*. The analyses include: a comparison of the effects of odors from four bivalve species on searching behavior of the crabs; a determination of the location of the principal chemoreceptors used in host location; and an analysis of

the importance of host induction ("olfactory conditioning": Thorpe and Jones, 1937; "ingestive conditioning": Wood, 1968; "host induction": Jermy, Hanson, and Dethier, 1968) in altering host preferences. This is discussed in light of the concept of chemical search images (Atema, 1977, 1980; Atema, Holland, and Ikehara, 1980).

MATERIALS AND METHODS

Subadult posthard stages (Pearce, 1964) and adult male and female specimens of *Pinnotheres maculatus* were collected from specimens of *Mytilus edulis* in the Woods Hole, Massachusetts, region. Their responses to odors from *Mytilus edulis*, *Argopecten irradians concentricus*, *Placopecten magellanicus*, and the ribbed mussel *Modiolus demissus* were tested. Of these four species, only *M. demissus* has never been reported as a host for *P. maculatus* (Williams, 1965).

The experimental apparatus was a plastic circular choice maze, modified from the design of Bartel and Davenport (1956) (Fig. 1). Odors were prepared for each species in 4-liter beakers by adding the number of live intact bivalves necessary to constitute 50 g of soft tissue wet weight per one liter of sea water. Odors and/or sea water dripped into the six radial chambers. This presented the crabs with six simultaneous stimuli from which they could make a choice. Each trial consisted of placing between six and thirty crabs in the central chamber. They were given 10 hr to enter the radial chambers, after which the trial was stopped and the crabs' distribution recorded.

Three types of tests were performed: control tests, where all six radial chambers received sea water; single-stimulus experimental tests, where odor dripped into only one of the radial chambers, and sea water into the other five chambers; and two-stimuli choice experimental tests, with four sea water radial chambers, and two chambers receiving odors, each odor from a different bivalve species. In choice experiments, the two chambers containing odors were always diametrically opposite each other. The apparatus was rotated 60° after each trial.

To determine the sites of the chemoreceptors used in host location, either antennules (A1) or antennae (A2) were ablated at least two days before the crabs were tested.

Host induction experiments were performed to analyze the degree of plasticity of the chemoresponses of these crabs. In these experiments, only adult crabs removed from *Mytilus* hosts were used. They were divided into two groups: induced crabs, which were placed in one-liter bowls with three individuals of *Argopecten*; and non-induced crabs, which were placed in identical bowls with sea water only. During the induction process, the water and scallops were changed two to three times per week, and crabs were free to crawl into or onto the scallops. The responses of these crabs in two-stimuli choice experiments (using *Mytilus* and *Argopecten* odors) were tested before induction and at weekly intervals for 5 weeks.

Two behavioral responses were analyzed for each trial run: chemically stimulated movement, and direct location of the odor source. Chemically stimulated movement (excitation) is defined as any upstream movement in response to the test solution and is measured as the percentage of crabs that entered any of the six radial chambers ("per cent excited"). It was not determined whether this movement was a rheotaxis, an increase in random movement, or both. Location of odor (attraction) is defined as the ability of crabs to find the source of the test solution and is measured as the percentage of all excited crabs that entered the chamber containing the odor ("per cent attracted").

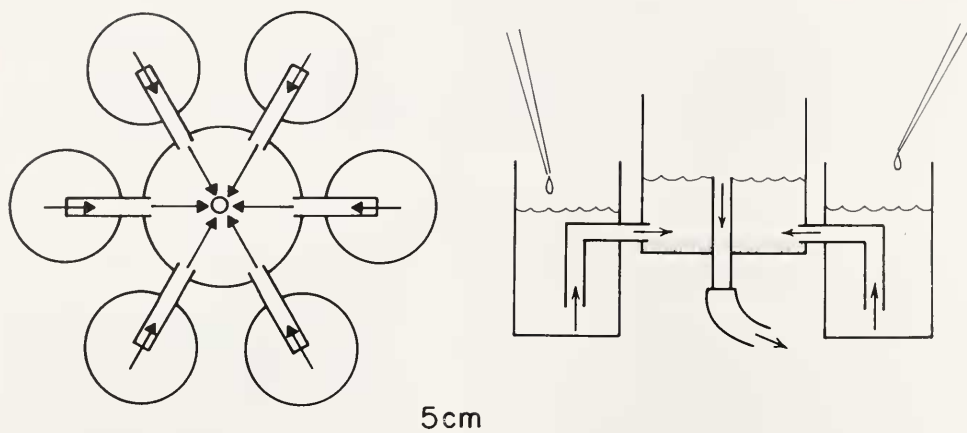


FIGURE 1. Circular choice maze. The maze consists of six radial chambers connected via L-tubes to a central chamber. The system was run under constant illumination, salinity (33‰), and temperature (14–18° C), with sterilized sand (3 hr at 290° C) covering the bottom of the central chamber. Water dripped into each of the radial chambers at a rate of 2 to 3 drops per sec. Water flowed from each of the radial chambers into a central chamber, which drained by a central standpipe. Arrows indicate the direction of water flow.

Statistical analyses are based on the assumption that the distribution of crabs in the radial chambers is related to their ability to sense a change in the chemical environment. Chi-square values were calculated for each trial to test if there was a preference for the chamber with the body odor. Heterogeneity Chi-square analyses were performed to check the validity of combining all the replicate trials within each treatment group so that an overall Chi-square test could be performed. All tests met this condition, except for the response to *Modiolus* odor, in which female crabs showed an attraction, but males and subadults did not. Chi-square tests were then performed for the compiled data using the 0.05 level of significance.

RESULTS

The control tests demonstrate that the crabs had no preference for any single radial chamber ($N = 140$, $\chi^2 = 8.96$, $P > 0.10$). Single-stimulus experimental tests show that intact crabs removed from specimens of *Mytilus* were highly attracted to *Mytilus* host odor and did not respond significantly to odor from *Argo-*

TABLE 1

Effects of bivalve odors on P. maculatus searching behavior.

Stimulus	% Excited	% Attracted	N	χ^2*	P
<i>Mytilus</i> odor	90.7	55.6	129	127.40	<0.001
<i>Placopecten</i> odor	85.3	19.7	95	0.56	>0.25
<i>Argopecten</i> odor	87.6	15.3	97	0.12	>0.50
<i>Modiolus</i> odor					
Female crabs	84.4	42.1	45	17.73	<0.001
Male and S crabs	70.2	17.0	67	0.01	>0.90

* Chi-square values determined by comparing number of crabs attracted to the radial chamber with bivalve odor and number of crabs attracted to the other five sea water radial chambers against the distribution expected from random movement. S = subadult posthard stage crabs.

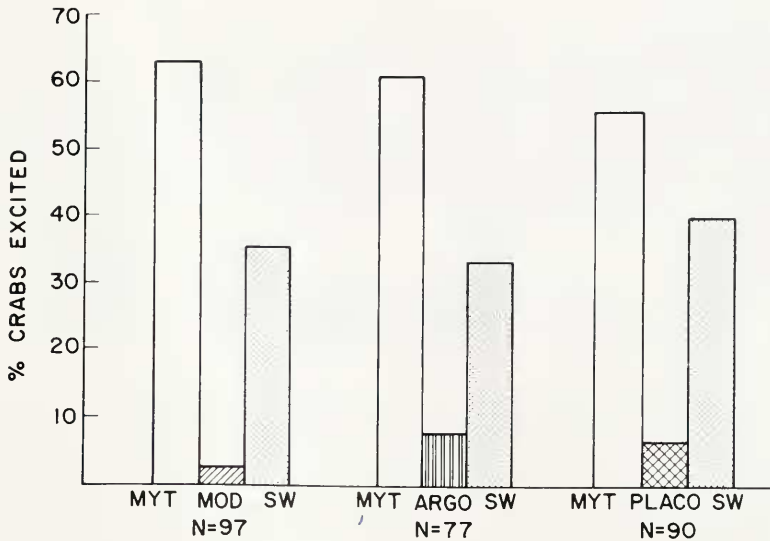


FIGURE 2. Preference of *P. maculatus* for *Mytilus* host odor in two-stimuli choice experiments. Chi-square tests show significant differences ($P < 0.001$) for all three tests in which the number of crabs attracted to *Mytilus* host odor and number of crabs attracted to odor from the other bivalve species are compared to the distribution expected from random movement. MYT = crabs attracted to *Mytilus* chamber; ARGO = crabs attracted to *Argopecten* chamber; PLACO = crabs attracted to *Placopecten* chamber; MOD = crabs attracted to *Modiolus* chamber; SW = crabs attracted to the four sea water chambers.

pecten or *Placopecten*; only the female crabs responded to *Modiolus* odor (Table I). In the two-stimuli choice experiments, crabs from *Mytilus* hosts strongly preferred *Mytilus* odor over odor from any other bivalve species tested ($P < 0.001$) (Fig. 2).

Ablation studies were performed to determine the sites of chemoreceptors used in host location. Trauma caused by appendage ablation is negligible, since intact crabs and crabs without antennules responded similarly in control tests: both groups show an equal degree of random movement (77.9 and 75.0% excitation, respectively), and both groups show random distribution in the six radial chambers ($P > 0.10$ and $P > 0.50$, respectively). Intact crabs and crabs without an-

TABLE II

Effects of ablations on chemically stimulated searching behavior.

Appendages removed	% Excited†	% Attracted	N	χ^2*	P
None	90.7	55.6	129	127.40	<0.001
Antennae (A2)	96.6	40.4	59	23.02	<0.001
Antennules (A1)	72.8	22.0	81	1.23	>0.25

Single-stimulus experiments, using *Mytilus* host odor as the stimulus.

* Chi-square values determined by comparing number of crabs attracted to *Mytilus* chamber and number of crabs attracted to the other five sea water chambers against the distribution expected from random movement.

† A one-way ANOVA with Scheffé's Multiple Comparison Procedure (using % excited values normalized by arcsine transformation) demonstrates that host odor caused increased movement (excitation) in intact and A2-less crabs, but not A1-less crabs ($P < 0.05$).

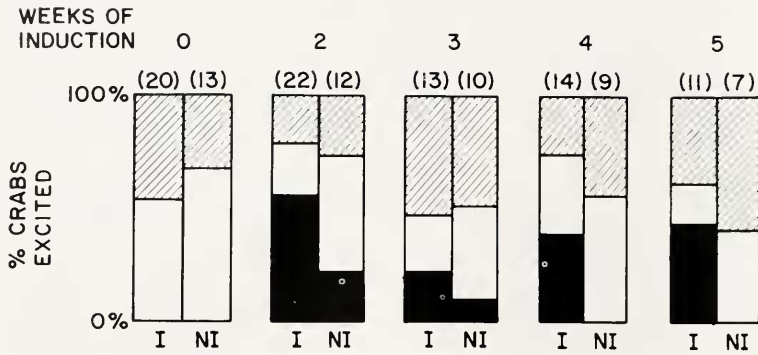


FIGURE 3. Induction of crabs from *Mytilus* to *Argopecten*. At weeks four and five, analysis by 2×2 contingency tables demonstrates a significant difference ($P < 0.05$) between number of induced crabs and number of non-induced crabs choosing the *Argopecten* chamber. Number in parentheses is the total number of crabs excited. I = Induced crabs; NI = Non-Induced crabs. Shaded areas represent crabs attracted to *Mytilus* chamber; open areas represent crabs attracted to the four sea water chambers; solid areas represent crabs attracted to *Argopecten* chamber.

tennae were significantly attracted to host odor, whereas the crabs without antennules were not (Table II). In addition, a one-way analysis of variance (ANOVA) demonstrates that host odor also caused an increased excitation in intact crabs and in crabs without antennae, but not in the crabs without antennules (Table II). Thus, antennules are the primary sites of chemoreceptors that detect *Mytilus* host odor, resulting in both increased general movement and source location.

Induction experiments were performed to explain why crabs found in *Mytilus* hosts do not respond to odors from other bivalves in which pea crabs are known to inhabit. These experiments (Fig. 3) reveal that experience with a new host significantly altered the olfactory response of adult crabs to the host odor. Non-induced crabs maintained a high preference for *Mytilus* odor, with little response to *Argopecten* odor. Induced crabs, on the other hand, showed an increased responsiveness to *Argopecten* odor after two weeks of induction. By the fourth and fifth weeks of induction, the difference in the responses of induced and non-induced crabs to *Argopecten* odor became statistically significant ($P < 0.05$). However, even with this increased response to *Argopecten* odor, a large proportion of the crabs still responded to the *Mytilus* odor.

DISCUSSION

This investigation demonstrates that there are chemoreceptors on the antennules of *P. maculatus* that elicit searching behavior in the presence of odor from the host, *Mytilus edulis*. These crabs, however, failed to respond to odor from two other bivalve species, *Argopecten irradians concentricus* and *Placopecten magellanicus*, and only the female crabs seemed to be attracted to *Modiolus demissus* odor (Table I). The latter observation cannot, at present, be explained. Also, in choice experiments, the crabs showed a strong preference for *Mytilus* odor over the odor from other potential hosts (Fig. 2). These results are different from those of Sastry and Menzel (1962), who found that *P. maculatus*, removed from *Argopecten*, is attracted to odor from both *Argopecten* and *Atrina*, and when given a choice between the two bivalves, is equally attracted to both. This discrepancy could

be explained by several factors. Different populations of pea crabs could be responding differentially to the same stimulus due to slight differences in olfactory receptors, as has been shown in the oriental fruit fly (Metcalf, Metcalf, Mitchell and Lee, 1979). Alternatively, odors of different populations of *Argopecten* may be qualitatively and/or quantitatively different (Bayne and Scullard, 1977), affecting the responses of the crabs. In addition, the odors of *Argopecten* and *Atrina* may be more similar to each other than are the odors of *Mytilus* and the other bivalves studied here.

Antennular chemoreceptors accounted for most of the response of the pea crabs to *Mytilus* host odor (Table II). Antennules are known by behavioral and neurophysiological studies to be important in host location for the commensal shrimp *Betaeus harfordi* and *B. macginitiae* (Ache and Case, 1969; Ache and Davenport, 1972; Ache, 1975). For pea crabs, hosts provide not only shelter but a constant food source; therefore, host odor might also be considered a feeding stimulus.

A plausible explanation for the observation that adult crabs show little attraction to odors from hosts which conspecifics are known to invade is that once a crab inhabits a host, it develops a stronger chemical attraction to the odor of that host. The induction experiments, designed to test this hypothesis, demonstrate that olfactory preferences of adults can be altered. After two weeks of induction to *Argopecten*, there was an increased responsiveness to *Argopecten* odor; this increase became statistically significant after four weeks (Fig. 3). Gray *et al.* (1968) reported changes in olfactory responses of the pinnotherid crab *Dissodactylus mellitae* to host odors after only one or two days of "acclimation" to a new host species. Modification of olfactory responses due to experience with a host or host odor has been reported for a variety of other symbiotic invertebrates (parasitic wasp *Nemeritis canescens*: Thorpe and Jones, 1937; tobacco hornworm *Manduca sexta*: Jermy *et al.*, 1968; polynoid polychaete *Arctonoe pulchra*: Dimock and Davenport, 1971).

The mechanism for such olfactory plasticity used in searching behavior could be described as chemical search image formation. The concept of search images was first discussed by von Uexküll (Uexküll and Kriszat, 1934), and later developed by Tinbergen (1960) to explain prey selection by the titmouse *Parus major*. Tinbergen described search image formation as a central perceptual change, involving improvement of the ability of the predator to detect the prey. As this bird is a visual predator, the term "visual search image" was adopted. Chemical search images are functionally similar, but are more important for animals relying on input from chemical senses, such as pea crabs. When the chemical search image of the animal is matched by the "chemical picture" of the environment, searching behavior may be initiated (Atema, 1977, 1980). It was recently shown that tuna change their olfactory excitability to specific prey odors depending on recent feeding experience. Modifiable chemical search images could describe the observed effects and appear useful in the tuna's behavioral ecology (Atema *et al.*, 1980). Home stream recognition by salmon (Scholz, Horrall, Cooper, and Hasler, 1976) could be considered a classical case of the use of a chemical search image.

This chemosensory plasticity would be adaptive considering that *P. maculatus* is a host-generalist, with fourteen host species reported (Williams, 1965). Although the chemosensory behavior of larval pea crabs is presently unknown, it might be expected that the "invasive stage" (Pearce, 1964, 1966) of this host-generalist symbiont can respond to a variety of chemical signals found in many potential hosts. Similar cases are known for other crustaceans: the settling stage

of the symbiotic brachyuran *Echinoecus pentagonus* responds to chemical cues from its host urchin *Echinothrix calanaris* as well as to odors from the closely related *E. diadema*; larval barnacles (*Balanus balanoides* and *Elminius modestus*) settle in response to proteins in the epicuticle of many barnacle and other arthropod species (Crisp and Meadows, 1962). After finding a host, *P. maculatus* can become conditioned to this host species, such that the response to the odor of this species increases. Such a change in olfactory response has been demonstrated here for adult specimens of *P. maculatus*. This phenomenon would be important in host reinvasion which can occur in various life stages. For example, there would be an enhanced likelihood of reinvasion by the hard stage following copulatory swarming, and by any stage following damage or death to the host.

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SUMMARY

1. The pea crab *Pinnothores maculatus* is a host-generalist symbiotic brachyuran. When subadult posthard and adult crabs are removed from the bivalve *Mytilus edulis*, *Mytilus* host odor increases their nonspecific movement as well as their location of the odor source. Odors from other previously recorded host species, however, generally do not affect their searching behavior.

2. Such response specificity may be due to olfactory induction to their host. Adult crabs removed from the blue mussel *Mytilus edulis* can be induced to respond to odor from the bay scallop *Argopecten irradians concentricus*. Such induction supports the plastic "chemical search image" concept.

3. Antennules are the principal sites of chemoreceptors used in host location.

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